Gastrointestinal Stromal Tumors in Children and Young Adults

A Clinicopathologic, Molecular, and Genomic Study of 15 Cases and Review of the Literature

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Summary: Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors of the intestinal tract that typically occur in adults over the age of 40 years. GISTs in younger patients are rare and not well characterized. The objective was to define the characteristics of GISTs in children and young adults (<30 years old). Clinicopathologic and molecular features, including *KIT/PDGFRA* genotype, in GISTs from 5 children and 10 young adults were analyzed. Gene expression analysis was performed on 5 gastric tumor samples from 2 children, 2 gastric tumors from young adults, and 10 gastric GISTs from older adults using an U133A Affymetrix platform (22,000 genes). All five pediatric GISTs occurred in girls, involved the stomach as multiple nodules, showed predominantly an epithelioid morphology, often involved lymph nodes, and lacked *KIT* or *PDGFRA* mutations. Although all five patients developed recurrence (four in the liver, three in the peritoneum, and two in both sites), four are still alive with disease. Of the 10 GISTs in young adults, half occurred in the small bowel and had spindle cell morphology, and one case had lymph node metastasis. *KIT* mutations were identified in seven cases, four in exon 11 and three in exon 9. Seven patients developed recurrence, and at last follow-up two patients had died of disease. Gene expression analysis showed high expression of *PHKA1*, *FZD2, NLGN4, IGF1R*, and *ANK3* in the pediatric and young adult versus older adult cases. GISTs that occur in children are a separate clinicopathologic and molecular subset with predilection for girls, multifocal gastric tumors, and wild-type *KIT/PDGFRA* genotype. In contrast, GISTs in young adults are a more heterogeneous group, including cases that resemble either the pediatric or the older adult-type tumors. The distinct gene expression profile suggests avenues for investigation of pathogenesis and potential therapeutic strategies.

Key Words: gastrointestinal stromal tumor, *KIT* mutation, receptor tyrosine kinase, gene expression, pediatric

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract in adults. GISTs are thought to originate from interstitial cells of Cajal (ICC) or an ICC precursor, since both have similar ultrastructural features and express the KIT receptor tyrosine kinase. In over 80% of cases, GISTs have activating mutations in *KIT* or *PDGFRA*, which encode class III receptor tyrosine kinases.[1,2] GISTs typically occur in adults over the age of 40, with a peak incidence in the sixth and seventh decades of life.[3] Although the clinicopathologic and molecular characteristics of GISTs in adults have been well characterized,[4–7] there are sparse data on GISTs in children and younger adults, in whom GIST occurs rarely.[8,9] A minority of GISTs in pediatric patients are associated with Carney's triad or neurofibromatosis type 1 and appear to have a different pathogenesis than the cases in older adults.[10,11] Anecdotal reports of sporadic pediatric GISTs suggest that an epithelioid morphology and wild-type *KIT* phenotype are common.[4,8] In comparison, most GISTs in older adults have spindle cell morphology and *KIT* mutations, usually involving the juxtamembrane domain.[1,7] GISTs in young adults (<30 years old) have not yet been studied separately. Our objective was to define the characteristics of GISTs in children and young adults.

METHODS

Clinicopathologic Analysis

Patients with GIST treated at Memorial Sloan-Kettering Cancer Center between 1982 and 2003 who were less than

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30 years old at diagnosis were identified from our prospective sarcoma database as well from the pathology database. Pediatric cases were defined as younger than age 18 years. In addition, we included young adult patients (older than 18 but less than 30) to test whether this group is related more closely to the pediatric or the adult subset, from the clinicopathologic and molecular aspects. Patient, tumor, and treatment data were obtained from the database. The pathologic diagnosis was confirmed using standard hematoxylin and eosin staining and CD117 immunostaining on formalin-fixed paraffin-embedded tissue, as previously described.[2] Immunohistochemistry was also performed for smooth muscle actin (SMA) and CD34 using prediluted mouse monoclonal antibodies (Ventana Medical Systems, Tucson, AZ). Smooth muscle and endothelial cells of the blood vessels were used as respective internal controls. Electron microscopy was performed using standard methods when adequate tissue was available.

KIT/PDGFRA Genotype

Mutation analysis was performed as described previously.[4] DNA was isolated in nine cases from snap-frozen tumor tissue samples stored at −70°C, using a standard phenol-chloroform organic extraction protocol. For the remaining six cases with only formalin-fixed paraffin-embedded tissue, DNA was obtained using the DNeasy Tissue Kit (Qiagen Inc, Valencia, CA). One microgram of genomic DNA was subjected to PCR using Platinum TaqDNA Polymerase High Fidelity (Life Technologies, Inc, Gaithersburg, MD). The PCR conditions were as follows: 94°C for 4 minutes, 94°C for 30 seconds, the relevant annealing temperature for each primer set for 30 seconds, and 72°C for 30 seconds (35 cycles); and 72°C for 3 minutes. The PCR products were identified by agarose gel electrophoresis using a 2% MetaPhor agarose gel (BioWhittaker Applications, Rockland, ME). The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) before sequencing. The sequencing reactions for each case were performed from both the forward and reverse directions. All of the cases were first subjected to a PCR reaction using primers for *KIT* exon 11. If a mutation was not detected, then *KIT* exons 9, 13, and 17 and then PDGFRA exons 12 and 18 were sequentially examined. The primers for *KIT* are as published.[4] Primers for *PDGFRA* exon 12 were TCCAGTCACTGTGCTGCTTC and GCAAGGGAAAAGGGAGTCTT at 54°C and for exon 18, ACCATGGATCAGCCAGTCTT and TGAAGGAGGATGAGCCTGACC at 55°C.

Hybridization of Affymetrix Oligonucleotide Chips

In five samples from two children and two samples from young adults, adequate frozen tissue was available for RNA extraction and gene expression analysis. All but one of the young adults (exon 9 mutation) were wild type for both *KIT* and *PDGFRA*. Since each tumor was located in the stomach, we used a group of 10 gastric GISTs occurring in older adults for comparison (8 of them were previously reported[12]). In the 10 adult samples, there were seven *KIT* exon 11 mutations, two *PDGFRA* mutations, and one wild type. None of these patients had received imatinib mesylate.

Gene arrays were used as described previously.[12] RNA was isolated using RNAwiz RNA Isolation Reagent (Ambion, Austin, TX), and samples were treated with RNase-free DNase (Qiagen) according to the manufacturer's instructions. Twenty-five to 50 nanograms of total RNA was tested for quality on an RNA 6000 Nano Assay (Agilent, Palo Alto, CA) using a Bioanalyzer 2100. RNA with an OD260/280 ratio greater than 1.8 was chosen for expression profiling experiments. Two micrograms of high-quality total RNA was then labeled according to protocols recommended by the manufacturer. Briefly, after reverse transcription with an oligo-dT-T7 (Genset), double-stranded cDNA was generated with the Superscript double-stranded cDNA synthesis custom kit (Invitrogen Life Technologies, Carlsbad, CA). In an in vitro transcription step with T7 RNA polymerase (MessageAmp RNA kit, Ambion), the cDNA was linearly amplified and labeled with biotinylated nucleotides (Enzo Diagnostics, Farmingdale, NY). Ten micrograms of labeled and fragmented cRNA was then hybridized onto a test array and a Human Genome U133A expression array (Affymetrix, containing 22,000 transcripts). Post-hybridization staining and washing were processed according to the manufacturer (Affymetrix). Finally, chips were scanned with a Hewlett Packard argon-ion laser confocal scanner.

Gene Array Analysis

The raw expression data were derived using Affymetrix Microarray Analysis 5.0 (MAS 5.0) software. The data were normalized using a scaling target intensity of 500 to account for differences in the global chip intensity. The expression values were transformed using the logarithm base 2. To find genes that associated with different GIST subsets, we applied filtering and statistical analysis constraints to the expression data to exclude those genes that did not vary significantly between comparison groups (less than two-fold change) or that were not expressed at high enough levels. A statistical group analysis was carried out to find genes that showed statistically significant differences in mean expression levels between the pediatric and adult-type GISTs. The log of the normalized expression data was analyzed using an unequal variance *t* test (Welch's approximation). To deal with the multiple testing problem, the false discovery rate (FDR)[13] method was used, and the list was cut off at an FDR of 0.05. The gene lists obtained for each individual analysis were cross-referenced against both the published literature and the gene ontology consortium database (http://www.geneontology.org/) using NetAffx (http://www.affymetrix.com). The data were clustered using two different methods and two gene lists. For one analysis the genes were filtered to contain only those that were scored present in at least 25% of the samples, which gave 12,092 genes. The data were then clustered using hierarchical clustering with the Pearson correlation metric and Ward linkage. To assess the robustness of the clustering result, bootstrap resampling was done.[14] A parametric method was used to resample the data to simulate noise. This was done 1,000 times and each replica of the data was clustered. The 1,000 trees were then combined using a majority rule algorithm[14] to give a consensus tree. Each node was scored by how many times it appeared in the 1,000 bootstrap trees.

The higher the score, the more robust the node. The data were also clustered using a gene list that selected for genes with significant fold changes between the groups. This time, hierarchical clustering with the Pearson metric and centroid linkage was used. We understand that by choosing the genes in this way, we are biasing the tree to separate the two groups. This cluster is shown for illustrative purposes to depict the distribution of expression values for genes significantly different in the two groups. The first clustering result is unbiased in its gene selection and the separation seen is a property of the data as a whole.

RESULTS

Demographics

From a total of 350 patients with GIST identified from our database, there were 5 children (1.4%). Each was a girl; age range at diagnosis was 10 to 15 years (Table 1). There was no clinical evidence of Carney's triad or history of neurofibromatosis in any of these cases. One patient (previously described[15]) was diagnosed simultaneously with an adrenal ganglioneuroma. There were 10 young adults with GIST, ranging in age from 18 to 29 years old; 7 were female.

Tumor Characteristics

The primary site of the tumor in all five pediatric cases was the stomach. The largest tumor size exceeded 5 cm in the three cases with available information. Strikingly, each patient had multiple tumor nodules in the gastrectomy specimen. Despite the presence of multifocal tumors, ICC hyperplasia was not present between the tumor nodules. Three of the tumors had a pure epithelioid morphology (Fig. 1), one showed mixed spindle and epithelioid cells, and one was predominantly spindled. The number of mitoses ranged from 2 to 48 per 50 high-power fields. All five tumors were positive for CD117 and negative for SMA, while four stained for CD34.

In the young adults with GIST, tumor size ranged from 3.5 to 18 cm. Five tumors (50%) were located in the small bowel, four were located in the stomach (40%), and one was extraintestinal. One patient, a 24-year-old woman (patient 10), had multifocal tumors. Another patient, a 29-year-old woman (patient 15), had multiple gastric masses identified on upper gastrointestinal series and diffuse intra-abdominal disease at the time of presentation. Surgical resection was not performed and multifocality could not be discriminated from peritoneal metastasis. Six tumors (60%) had a spindle cell morphology (four in the small bowel, two in the stomach), three (30%) showed mixed spindle and epithelioid features (one gastric, one small bowel, one intra-abdominal GIST), and only one tumor showed a pure epithelioid morphology (involving the stomach). All 10 tumors were positive for CD117. Four (40%) of them were also positive for CD34 and only one was positive for SMA.

Ultrastructural analysis on four pediatric and two young adult patients revealed cells with consistent long cell processes, either filopodia type, resembling anemone structures, or interdigitating cell processes, reminiscent of a neural proliferation (Fig. 2A). In two cases, dense core neurosecretory-type granules were present, and in one gastric tumor, short-spacing collagen "skeinoid fibers" were identified (see Fig. 2B). The electron microscopic findings for each tumor are summarized in Table 2.

TABLE 1. Clinical and Pathologic Characteristics of GISTs in Children and Young Adults

Case #	Age	Sex	Primary Site	Multi-focal	Size (cm)	Type	MF/50 HPF	IHC			KIT Mutation	Follow-Up (mos)	Time to Recurrence		Status
								KIT	CD34	SMA			Liver	Peritoneal	
1^	10	F	Stomach	Yes	14	E	6	+	+	−	WT	24	–	20	AWD
2*	10	F	Stomach	Yes	NA	S	6	+	−	−	WT	148	136	130	AWD
3*	12	F	Stomach	Yes	NA	E	3	+	+	+, W	WT	80	48	–	AWD
4*^	12	F	Stomach	Yes	8	E+S	2	+	+	−	WT	36	3	36	AWD
5	15	F	Stomach	Yes	9	E	48	+	+	−	WT	138	49	48	DOD
6^	18	F	Stomach	No	NA	E	NA	+	−	−	Exon 9	77	20	–	AWD
7	20	F	Duodenum	No	10	S	1	+	−	−	Exon 11	60	–	–	NED
8	23	M	Duodenum	No	18	S	1	+	+	−	Exon 11	29	10	At Dx	DOD
9	23	M	Jejunum	No	3.5	S	19	+	+	−	Exon 9	4	–	–	NED
10	24	F	Stomach	Yes	6.5	E+S	6	+	+	−	WT	151	120	40	AWD
11	24	F	Small bowel	No	12	S	1	+	−	+	Exon 9	148	–	120	AWD
12^	26	F	Stomach	No	NA	S	NA	+	−	−	WT	180	168	96	AWD
13	27	M	I-abd	No	NA	E	103	+	−	−	Exon 11	36	32	NA	DOD
14	28	F	Ileum	No	7.5	E+S	1	+	−	−	Exon 11	48	–	44	AWD
15	29	F	Stomach	No	NA	S	2	+	+	−	WT	2	–	At Dx	AWD

NED, no evidence of disease; AWD, alive with disease; DOD, dead of disease; I-abd, intra-abdominal; WT, wild type;
W, weak, MF, mitotic figures; HPF, high-power field; E, epithelioid; S, spindle, IHC, immunohistochemistry; SMA, smooth muscle actin.
*Case 2, previously reported in reference 15, was diagnosed synchronously with a calcified adrenal ganglineuroma; case 3 was previously reported in reference 31; case 4 was previously reported in reference 9.
^Cases subjected to gene expression analysis.

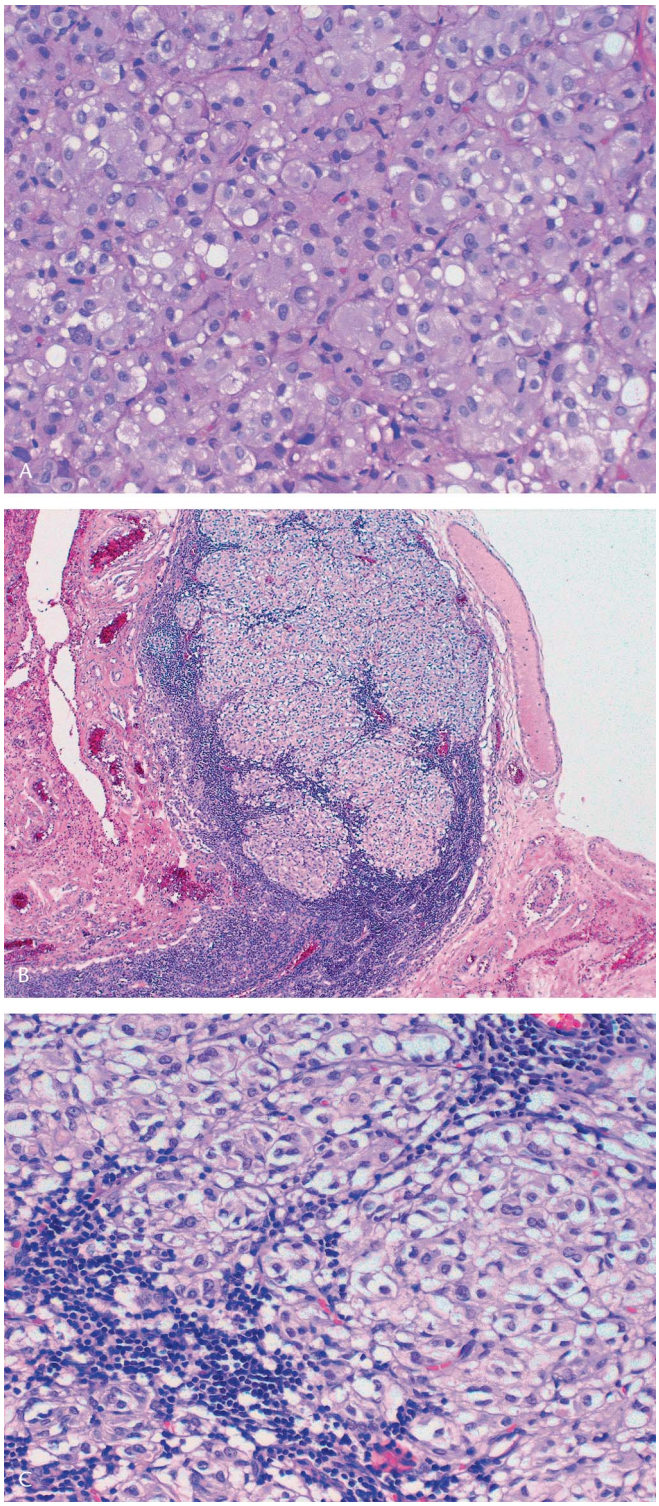

FIGURE 1. Microscopic appearance of a pediatric gastric GIST showing a pure epithelioid appearance. A, In the primary lesion the cells have a striking plasmacytoid appearance, with abundant eosinophilic cytoplasm (patient 3; H&E; ×200). B, C, Metastatic to a regional lymph node (patient 2; H&E, ×40, and inset ×400).

Mutation Analysis

None of the 15 patients had a detectable *PDGFRA* mutation in exon 12 or 18. There were no *KIT* mutations in the five children. In contrast, of the 10 tumors in young adults, only 3 (30%) were *KIT* wild type, while 3 (30%) had an exon 9 mutation and 4 (40%) had an exon 11 mutation (Table 3).

Gene Expression Analysis

We then investigated the gene expression profile of seven samples from two children and two young adults and compared them to the profiles of 10 gastric GISTs from older adults we had in part reported previously. A separate comparison between pediatric tumors only or young adult tumors only was not possible due to the limited number of samples available. First, an unsupervised clustering analysis using the bootstrap[14] was performed. All except one tumor

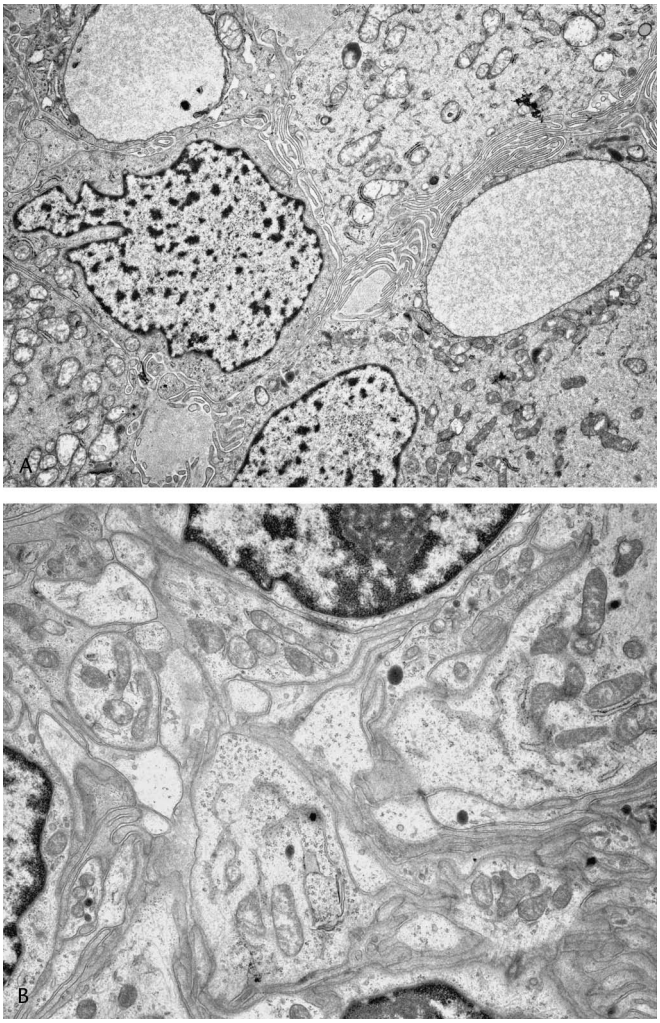

FIGURE 2. Ultrastructural appearance of a pediatric GIST. A, Low-power view of epithelioid cells separated by anemone-type cell processes and also by extracellular aggregate of skeinoid fibers; also note the large empty subnuclear vacuoles (patient 3; ×13,640). B, Detail of interdigitating cytoplasmic cell processes and dense core neurosecretory-type granules (patient 2; ×24,850).

TABLE 2. Electron Microscopic Features of GISTs in Children and Young Adults

Case # *	Type	Cell Processes	Skeinoid Fibers	Cell Junctions	Actin Filaments	Neuro-secretory Granules	Micro-tubules	Intermediate Filaments
1	E	Long filopodia "anemone"-like	−	−	−	−	−	−
2	S	ICP +++ neural-like	−	+	−	++	+	−
3	E	Long filopodia "anemone"-like	++	−	−	−	−	−
5	E	ICP, rare short microvilli-type	−	+	+/−	−	−	+
7	S	ICP ++ neural-like	−	+	+/−	+	+	−
8	S	ICP+	−	−	+/−	−	−	−

*Case number corresponds to the case number in Table 1.
ICP interdigitating cell processes; CP, cell processes.

(patient 4) clustered within the pediatric and young adult group (Fig. 3A). Using an unequal variance *t* test with an FDR of 0.05, 385 differentially expressed genes were found between the two groups (Table 4). Among them, the neuronal cell adhesion molecule, neuroligin 4 (*NLGN4*) and ankyrin 3 (*ANK3*), frizzled 2 (*FZD2*), insulin-like growth factor receptor (*IGF1R*), and phosphorylase kinase alpha 1 were upregulated in the pediatric and young adult samples. In contrast, *DPT* (dermatopontin), *PDGFRA*, *RAB38*, a member of the RAS-family, and G-protein-coupled receptor 88 (*GPR88*) were highly expressed in the older adult gastric tumor as opposed to the pediatric one. In parallel, a hierarchical clustering was performed using the GeneSpring 6.1 software using a total of 1,779 genes differentially expressed (fold change >2, standard correlation) between the two groups. A similar clustering was obtained, with one of the two samples from pediatric patient 4 grouping separately from the other pediatric and young adult cases (see Fig. 3B).

Clinical Outcome

Follow-up data were available in all cases (see Table 1). In the pediatric group, after a median of 80 (range 24–148) months, four of the five patients developed recurrent disease in the stomach requiring completion gastrectomy. Lymph node dissection was performed in four cases and showed lymph node metastasis in three cases (see Fig. 1). In two of these three cases (patients 2 and 4), there was no evidence of tumor in the lymph nodes at the time of the primary resection, but lymph node metastases were identified upon resection of the recurrent tumor within the stomach. Three patients developed recurrences both in the liver and intra-abdominally, and one patient each either in the liver or peritoneal cavity. From the four patients with liver metastases (diagnosed 3–136 months from presentation), one died of disease and the remaining three are alive with disease 12, 32, and 33 months since developing liver metastases.

Clinical follow-up for the 10 young adult patients ranged from 2 to 180 months, with a median of 54 months. Lymph node metastasis was present in one patient. Two patients died of disease with metastasis to the liver at 29 and 36 months from the initial diagnosis; two are alive with no evidence of disease at 4 and 60 months of follow-up; and the remaining six are alive with disease (two with metastatic disease involving the liver, three with abdominal disease, and one with metastasis involving the liver as well as abdominal disease). Two of the patients with *KIT* exon 11 mutations died of disease after a follow-up of 29 and 36 months. Of the three patients with exon 9 mutations, two are alive with disease after a follow-up of 77 and 148 months, and the third patient has no evidence of disease after a short follow-up of 4 months.

Imatinib Therapy

Two of the children were treated with imatinib mesylate, an oral molecular inhibitor of KIT, for their recurrence. One had extensive liver and intra-abdominal recurrence and was treated for 4 months without response and died soon thereafter. The second patient had intra-abdominal recurrence and has stable disease 12 months since treatment started.

Imatinib therapy was administered to 6 of the 10 young adult patients: 4 for recurrent disease and 2 as an adjuvant after resection of the primary. Of the four with recurrent disease, one had repeated intratumoral hemorrhage necessitating termination of therapy and the other three have stable disease 5 to 12 months later.

DISCUSSION

GISTs represent the great majority of tumors formerly diagnosed as leiomyomas, leiomyosarcomas, and leiomyoblastomas. GISTs generally occur after age of 40, with a median

TABLE 3. KIT Exon 11 Deletions and Point Mutations in Pediatric and Young Adult GISTs

	550	560	570	580	590
Wild type	K P M Y E V Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L	S F G
Case 7	K – – – – – Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L	S F G
Case 8	K P M Y E V Q – – V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L	S F G
Case 13	K P M Y E V Q W K V	– E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L	S F G
Case 14	K P M Y E V Q W K D	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L	S F G

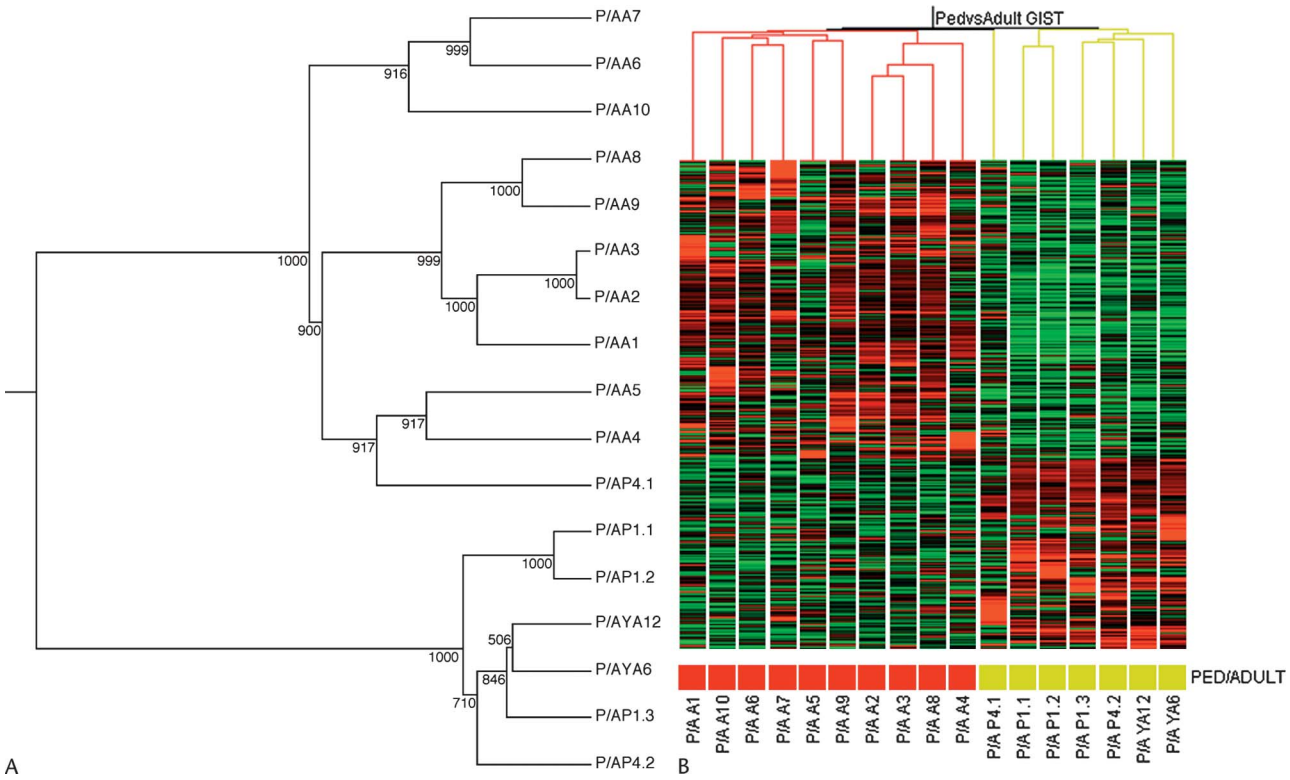

FIGURE 3. Gene expression analysis of 7 pediatric and young adult samples compared with a control group of 10 gastric adult GISTs. A, Hierarchical clustering with genes filtered to be those present in at least 25% of the samples, using the Pearson correlation metric (distance = (1/2)(1 - p), where p is the correlation) ward linkage. A consensus tree was built from 1,000 bootstrap samples. The number at each node represents how many times that subtree occurred. B, Hierarchical clustering using Genespring 6.1 based on 1,776 genes, filtered by flag, expression, and fold change >2 with the Pearson correlation metric and centroid linkage. P, pediatric; YA, young adult; A, older adult; P/A: pediatric and young adult vs. older adult groups comparison.

varying from 55 to 65 years, and the tumor is slightly more common in men.[3] In adults, the tumor occurs most commonly in the stomach (two thirds of cases), followed by the small bowel and rectum. The proportion of patients under the age of 40 is approximately 5% in the larger clinicopathologic series.[16] GIST is extremely rare in children: only 12 sporadic cases have been reported to date.[8,9,17–21] Most of the reported pediatric GISTs lack KIT immunohistochemistry and/or molecular analysis for *KIT/PDGFRA* genotyping.

A number of conclusions can be made regarding pediatric GIST based on our data and the published cases (Table 5). In all 15 children, the tumor originated in the stomach, and there was a striking female predominance (all but two occurred in girls). The majority (11/15 [73%]) of pediatric GISTs seemed to be associated with predominantly epithelioid or mixed epithelioid and spindle cell morphology. Our findings support the initial observations by Kerr et al,[8] reporting on the largest series to date, four girls (age range 10–16 years) with epithelioid gastric tumors. In addition, Kerr et al made an interesting observation regarding the multifocal distribution of these tumors within the gastrectomy specimen, with the presence of nodules of variable number and size separated by normal gastric tissue. We specifically analyzed our cases for the presence of multifocality and multinodularity, carefully reviewing the operative notes, gross reports, and slides, and validated their observation: all five cases were described grossly as multifocal or multinodular. This finding has significant clinical relevance, since the presence of multifocal gastric disease was most likely responsible for "local recurrence" within the gastric stump in four of five patients, a phenomenon uncommon in the adult population.

Another provocative finding in the series by Kerr et al was the presence of metastases to regional lymph nodes in two of their patients. This phenomenon was present in three of our five pediatric patients. This pattern of metastasis is extremely uncommon in adults with GIST but was seen in 1 of the 10 young adults in our study.

The presence of multifocal disease identified in children with GIST raises the question of a possible relationship with other syndromes typically associated with multiple GISTs, such as familial GIST syndrome, Carney's triad, and neurofibromatosis type I (NF1). The patients with familial GISTs have inherited germline mutations in *KIT* or *PDGFRA*,[22,23] but the pathogenesis of GIST in the two latter syndromes is unknown. GISTs in patients with NF1 have been recently shown to lack the *KIT* mutation.[11] GISTs associated with Carney's triad typically occur in a younger age group and with a female predominance and include one or two additional tumors, such as extra-adrenal paraganglioma and pulmonary chondroma. Interestingly, both familial and NF1-related

TABLE 4. Genes Expressed Differentially in Children and Young Adults Versus the Older Adult Control Group

Gene Symbol	Gene Name	*P* Value	FC	Location	Molecular Function (GO)
PHKA1	phosphorylase kinase, alpha 1 (muscle)	4.46E-09	3.76	Chr:Xq12-q13	phosphorylase kinase, intrinsic regulator activity
ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	4.38E-07	−12.54	Chr:12p12.1	sulfonylurea receptor activity
RAB38	RAB38, member RAS oncogene family	5.04E-07	−22.28	Chr:11q14	protein transporter activity
MITF	microphthalmia-associated transcription factor	9.56E-07	2.24	Chr:3p14.1	
FZD2	frizzled homolog 2 (Drosophila)	2.05E-06	12.22	Chr:17q21.1	non G-protein coupled 7TM receptor activity
ASRGLI	asparaginase like 1	2.92E-06	14.89	Chr:11q12.3	asparaginase activity
NLGN4	neuroligin 4	9.49E-06	16.71	Chr:Xp22.33	cell adhesion molecule activity
GPRC5B	G protein-coupled receptor, family C, group 5, member B	5.14E-05	6.22	Chr:16p12	metabotropic glutamate, GABA-B-like receptor activity
CDKN2A	cyclin-dependent kinase inhibitor 2A (p16, inhibits CDK4)	5.37E-05	2.50	Chr.9p21	cyclin-dependent protein kinase inhibitor activity
FOXDI	forkhead box D1	6.35E-05	13.13	Chr:5q12-q13	transcription factor activity
DPT	Dermatopontin	0.835E-04	−36.19	Chr:1q12-q23	cell adhesion
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	1.055E-03	−9.39	Chr:4q11-q13	transmembrane receptor protein tyrosine kinase signaling pathway
IGF1R	insulin-like growth factor 1 receptor	1.396E-03	14.22	Chr:15q25-26	
GPR88	G-protein coupled receptor 88	0.0001703	−16.32	Chr:1p21.3	G-protein coupled receptor activity
ANK3	ankyrin 3, node of Ranvier (ankyrin G)	0.0001205	10.56	Chr:10q21	structural molecule activity
GLIPR1	GLI pathogenesis-related 1 (glioma)	0.000174	−6.00	Chr:12q21.1	

Selective discriminatory genes are listed according to their gene designation, p value, fold change (FC) pediatric and young adult vs. older adult group, and molecular function.

GISTs show patchy ICC hyperplasia, which has not been observed in sporadic pediatric cases.[22] Otherwise, the cases reported to date within the spectrum of Carney's triad bear a close resemblance to the sporadic pediatric GISTs, which are typically located in the stomach, multifocal, epithelioid, and often associated with regional lymph node metastases.[10,24] Moreover, the relatively long survival of patients with Carney's triad, even in the presence of lymph node or liver metastatic disease,[10] closely resembles the biology of the sporadic pediatric GISTs. As noted in the children described here, the multiple gastric tumors seen with Carney's triad also required multiple gastric operations, which ended with total gastrectomy for their eradication. Like patients with Carney's triad, there is no evidence of familial disease identified in "sporadic"

TABLE 5. Current Data and Review of Literature on Sporadic Pediatric GISTs*

Series	Case #	Age	Sex	Location	Morphology	IHC Profile			KIT Mutation	Outcome
						CD34	SMA	KIT		
Current report	1	10	F	Stomach	E	+	−	+	WT	AWD 24 mos
	2	10	F	Stomach	S	−	−	+	WT	AWD 148 mos
	3	12	F	Stomach	E	+	+,W	+	WT	AWD 80 mos
	4	12	F	Stomach	E + S	+	−	+	WT	AWD 36 mos
	5	15	F	Stomach	E	+	−	+	WT	DOD 138 mos
Kodet et al[17]	6	15	F	Stomach	S	ND	−	ND	ND	AWD 10 mos
Perez-Atayde et al[18]	7	16	F	Stomach, duodenum	E + S	ND	−	ND	ND	NED 30 mos
Oguzkurt[19]	8	13	F	Stomach	E + S	ND	−	ND	ND	NED 12 mos
Kerr et al[8]	9	16	F	Stomach	E + S	+	–	ND	ND	NED 9 yrs
	10	13	F	Stomach	E + S	+	–	ND	ND	NED 8 mos
	11	11	F	Stomach	E + S	+	–	ND	ND	AWD 23 mos
	12	10	F	Stomach	E + S	+	−	ND	ND	NED 105 mos
Budzynski et al[21]	13	14	F	Stomach	NA	+	−	+	ND	NED (duration of follow-up NA)
Haider et al[20]	14	10	M	Stomach	E + S	+	−	+	ND	NED 5 yrs
	15	11	F	Stomach	S	−	−	−	ND	NED 20 mos

*This list does not include pediatric GISTs occurring within Carney's triad, congenital GISTs, or KIT-negative GISTs (see text for more details).
E, epithelioid; S, spindle, W, weak; ND, not done; WT, wild type; AWD, alive with disease; NED, no evidence of disease; NA, not available; DOD, dead of disease.

pediatric GISTs; therefore, some of the latter cases may be a *form fruste* of this syndrome.

It is uncertain whether congenital GISTs exist and how they may be related to pediatric GISTs. Most older reports designate congenital stromal tumors as either intestinal leiomyosarcomas or solitary intestinal fibromatosis.[25,26] They most commonly arise in the jejunum and have a female predominance.[25] More recent reports[27,28] have suggested that neonatal or congenital stromal tumors have a similar morphologic appearance as adult GISTs. Wu et al[28] reported a KIT- and CD34-immunopositive, 3.5-cm jejunal mass producing complete bowel obstruction in utero at 39 weeks gestation in a male fetus. In contrast, the case reported by Bates et al,[27] a term female infant with a 1.5-cm jejunal nodule, lacked KIT or CD34 immunoreactivity support for a GIST diagnosis. A recent report by Jeng et al[29] of a 2-year-old girl displaying congenital ICC hyperplasia restricted to the cecum brings another level of complexity to this subject. The ICC hyperplasia was confirmed by positivity for both KIT and CD34 and was noted in the absence of a GIST or germline or somatic *KIT* mutation. As adult and pediatric GISTs originate or differentiate toward an ICC phenotype, it is expected that GISTs occurring in neonates should be KIT positive, since normal ICCs are KIT positive at birth.[30] Until more cases are identified, the existence of congenital GISTs remains unclear.

The uniform immunoprofile and consistent ultrastructural findings seen in our patients matched the findings described in the four patients of Kerr et al.[8] None of the tumors in either series showed convincing evidence of muscle differentiation by immunohistochemistry or electron microscopy. In contrast, most tumors were CD34 positive and ultrastructurally showed a neural-like phenotype, with long cell processes, some resembling anemone cell features.[31] Dense core neurosecretory-type granules were identified in all four patients studied by Kerr et al[8] and but were seen in only one of our patients, in association with microtubules. These features were suggestive of a GANT, an ultrastructural variant of GIST. Skeinoid fibers were also present in one of our patients but were absent in all of those in the Kerr et al[8] series.

In contrast to Kerr et al,[8] who suggested that pediatric GISTs were less aggressive because metastasis occurred in only one patient, four of our five patients developed liver metastases. Only one of our patients has died and the remaining three are alive after a long follow-up in the absence of imatinib therapy, suggesting an indolent post-recurrence course. This indolent clinical course suggests a different biology of pediatric GISTs compared with their adult counterparts. Of the two children treated with imatinib for recurrent disease, one had no response and the other had stable disease after 12 months of treatment. More data on pediatric patients will be necessary to determine whether imatinib achieves a comparable partial response or stable disease in over 75% of patients, as it does in adults.[32]

In adult GISTs, the overall incidence of *KIT* mutations varies from 65%[33] to 92%,[7] and it is also present in a significant number of incidental GISTs.[4] The majority of these mutations have been found in the juxtamembrane domain, in the hot-spot region of exon 11. Less frequently, *KIT* mutations occur either in the extracellular domain (exon 9) or the kinase domain (exon 13).[6,34] All five pediatric GISTs analyzed in this study failed to show *KIT* or *PDGFRA* activating mutations, suggesting a yet undefined pathogenesis for GIST in this population. This may also render children with GIST less responsive to imatinib, since GIST with wild-type *KIT* responds infrequently.[35] In contrast, 70% of young adult GISTs in this series had *KIT* mutations, almost equally divided between exon 11 or exon 9.

In comparison to the pediatric GISTs, only 4 of the 10 young adult GISTs were present in the stomach, and only 2 tumors had a predominantly epithelioid morphology. There was no correlation between anatomic site and morphology; four of the six tumors with spindle cell morphology involved the small intestine and two involved the stomach. In two young adult patients the tumors shared some characteristics with pediatric GISTs. These were two female patients, with epithelioid tumors involving the stomach. One of these tumors was multifocal and did not have a *KIT* or *PDGFRA* mutation. It is possible that in this case the tumor developed during childhood but was diagnosed at a much later age. The second case showed lymph node metastasis similar to the pediatric GISTs, but it had an exon 9 *KIT* mutation. The clinical outcome in the 10 young adult cases, including liver metastasis (50%) and intra-abdominal recurrence (70%), was similar to that seen in older adults.

The characteristic gene expression signature of GISTs includes high expression of KIT, G protein-coupled receptor (GPR20), protein kinase C θ (PKCθ), and other genes, which distinguishes them from other soft tissue sarcomas.[12,36–38] In a recent report, we observed that gene expression profiles in adult GISTs were heterogenous and were distinguished by both *KIT* genotype and anatomic site.[12] Differentially expressed genes were also found between germline and sporadic GISTs. With the current data, we identified transcriptional heterogeneity between the pediatric and young adult GIST samples compared with an adult GIST control group. Although all the samples under investigation had the same anatomic location, they differed in regard to their genotype; most of the pediatric lesions were wild type, while the adult control cases were predominantly *KIT* exon 11 mutations. Despite this intrinsic heterogeneity, a significant number of genes were found to separate between the two subsets. The two samples from young adult patients clustered with other pediatric samples rather than the older adult samples. The same two patients showed overlapping pathologic and clinical features with the pediatric subset, including female sex, gastric location, epithelioid phenotype (one case), and wild-type genotype (one case). Since all reported pediatric cases have a wild-type genotype, a better understanding of their gene expression profile might provide insight into the pathogenesis of these tumors. A number of potential candidate genes and therapeutic targets were found to be overexpressed in the pediatric subset, including *PHKA1, FZD2, CDKN2A, ASRGL1*, and *IGF1R* (see Table 4). *PHKA1*, the gene that was the most differentially expressed in this study, was previously reported as a candidate gene involved in a subset of AML and preleukemia in elderly women.[39] In contrast, *PDGFRA* was found to be one of the significantly overexpressed genes in the older adult group. Further studies are needed to test the role of these genes in pediatric GISTs.

In summary, pediatric GISTs are a distinct subset of this rare sarcoma, with strong predominance for girls, multifocal gastric location, and a wild-type phenotype. Lymph node

metastasis and local recurrence to the gastric stump is common in this setting. Despite a high recurrence rate, both in the liver and elsewhere within the abdomen, the clinical behavior of pediatric GISTs appears more indolent, even in the absence of imatinib therapy, compared with adult GISTs. In contrast, GISTs in young adults are more heterogeneous, with features of tumors from children or older adults.

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